

**DEMOGRAPHY, POPULATION GENETICS, AND SYSTEMATICS OF
HUACHUCA TREE FROGS (*HYLA WRIGHTORUM*): TAXONOMIC AND
CONSERVATION IMPLICATIONS**

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Erik W. A. Gergus, Principle Investigator

Department of Biology, Arizona State University,

Tempe, AZ 85287-1501

Tod Reeder, Co-PI

Department of Biology, San Diego State University,

San Diego, CA 92182

Brian K. Sullivan, Co-PI

Department of Life Sciences, Arizona State University West

P.O. Box 37100, Phoenix, AZ 85069-7100

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INTRODUCTION

The Mountain Treefrog, *Hyla eximia*, and Arizona Treefrog, *H. wrightorum*, inhabit meadows in pine-oak or pine-fir forests, or near slow-moving streams, generally at elevations above 5000 feet (Duellman, 1970; Stebbins, 1985). Based upon the taxonomy of Taylor (1938), *Hyla wrightorum* is allopatrically distributed with populations occurring relatively continuously along the Mogollon Rim of central Arizona into western New Mexico, the Huachuca Mountains and adjacent Canelo Hills of southeastern Arizona, and the Sierra Madre Occidental of México (Figure 1). *Hyla eximia* is found throughout the southern part of the Mexican Plateau, the Sierra Madre Oriental, and the Cordillera Volcánica in central México (Duellman, 1970). Throughout their distributions, Arizona and Mountain tree frogs are generally bright green above and possess a dark brown stripe that begins on the snout and passes through the nostril, eye, and tympanum to extend onto the flank (Duellman, 1970; Figure 2). Dorsal markings are highly variable with most individuals exhibiting some degree of spotting.

Considerable controversy has persisted as to whether *Hyla wrightorum* should be recognized or synonymized with *H. eximia* (e.g., Duellman, 1970; Renaud, 1976). *Hyla eximia* was described by Baird (1854) with a type locality from “Valley of México,” (Distrito Federal), México. *Hyla wrightorum* was diagnosed by Taylor (1938) as a species separate from *Hyla eximia* based on the presence in the former of larger size, anterior edge of tibia with heavy brown spots and lacking a white line, and proportionately longer legs. The contact zone between *H. eximia* and *H. wrightorum*, according to Taylor (1938), is somewhere in Chihuahua and Sonora, México, with *H.*

wrightorum being distributed north into Arizona and New Mexico. Schmidt (1953) arbitrarily listed *H. wrightorum* as a subspecies of *H. eximia*, although Blair (1960) provided evidence from mating calls indicating subspecies designation was premature and apparently incorrect. Jameson et al. (1966) recognized *H. wrightorum* as a subspecies of *H. regilla* (Pacific Treefrog) based on a multivariate discriminant function analysis of ten morphological measurements. Duellman (1970), however, synonymized *H. wrightorum* with *H. eximia* based upon similarity in tadpole morphology, adult morphology, and mating calls. Although Duellman (1970) contended that a mosaic pattern of variation exists in advertisement calls of *H. eximia*, he failed to take into account size and temperature of recorded individuals when analyzing geographic variation in calls. In light of Blair's (1960) identification of "fast" and "slow" *eximia* (pulse rates of advertisement calls at similar recording temperatures were dramatically different between some samples in southern México), Duellman's taxonomic conclusions are rendered suspect. Maxson and Wilson (1974) compared serum albumins of *H. eximia*, *H. regilla*, and *H. wrightorum* and supported Duellman's (1970) contention that *H. eximia* and *H. wrightorum* are closely related and together relatively divergent from *H. regilla*, a conclusion in contradiction with the results of Jameson et al. (1966). Renaud (1977) subsequently compared morphometric, allozyme, and advertisement call variation of Mogollon Rim and mainland México populations and concluded that the Arizona populations could be diagnosed from those in México based on differences in size (snout vent length, or SVL), shape, and dominant frequency of male advertisement calls. Based on these differences, Renaud (1977) referred the tree frogs of central Arizona and western New Mexico to *H. wrightorum* and those of mainland México to *H.*

eximia. Perhaps due to the fact that Renaud's (1977) results were not published in a peer-reviewed journal, his conclusions have largely been ignored or gone unnoticed (e.g., Stebbins, 1985; Collins, 1997).

Several aspects of previous taxonomic analyses (e.g., Duellman, 1970; Renaud, 1977) are questionable, and thus a reassessment of the taxonomic status of *Hyla eximia* and *H. wrightorum* is warranted. For instance, Renaud (1977) did not find any statistically significant relationship between dominant frequency (DF) and pulse rate (PR) against wet and dry-bulb air temperatures, water temperature, or SVL, hence no correction factors were applied in analyses of call data. However, Sullivan (1986) found a significant difference in mean pulse rate of frogs he recorded at Baker Lake, Arizona as compared to those of Renaud (1977) leading him to conclude that pulse rate may be influenced by body temperature, contrary to Renaud's (1977) assertion. Additionally, Sullivan (1986) recorded a statistically significant relationship between DF and SVL, contrary to Renaud (1977). Although Duellman (1970) based his synonymy of *H. wrightorum* with *H. eximia* largely on their similar advertisement calls, he did not report temperatures or SVLs of recorded individuals. By not taking into account temperature or size related phenomena, Duellman's conclusions also are questionable.

The taxonomic status of Arizona tree frogs in the Huachuca Mountains and adjacent Canelo Hills (hereafter referred to as Huachuca tree frogs) has not been investigated, and little is known about their distribution and abundance. The conservation and management of biodiversity is difficult when the taxonomic status of populations is unknown; knowledge of genetic and taxonomic diversity is often antecedent to the formulation of a conservation strategy or comparative evolutionary

studies (Vane-Wright, 1996; Vogler and DeSalle, 1994). Also, the viability of the Huachuca tree frog populations is of special concern because of their small, restricted nature; the extremely limited breeding activity exhibited by Huachuca tree frogs (Stebbins, 1985; Gergus, personal observation), as well as the presumably limited effort that has been given to searching for such populations, leaves open the possibility that more exist and wait to be discovered. Small populations have the potential for lower levels of genetic heterozygosity and increased potential for inbreeding depression, which may be harmful to the population, and small populations are also more susceptible to local extinction from unpredictable changes in the environment.

In recent years declines in amphibian populations worldwide have received increased attention (Blaustein and Wake, 1990; Blaustein et al., 1994). In some cases, strong evidence exists for human alteration of habitats attributing to declines, but in other instances, natural population fluctuations may give the appearance of significant declines (Pechmann, 1991). Species with restricted distributions may be prone to extinction simply because of their increased vulnerability to minor alterations or perturbations to their habitats or populations. Also, the possibility exists that species or populations may go extinct without knowledge of their existence, either because of their extremely limited distributions, or because little is known about their true taxonomic status, as in the case of cryptic species (Daugherty et al., 1990). Populations of Huachuca tree frogs may be prone to these factors because of their restricted geographic distribution and apparent small population sizes; baseline demographic data is necessary to successfully monitor populations fluctuations and to identify reasons for changes in demographics.

Recent theoretical and empirical advances in species analysis have improved the rigor with which taxonomic analyses are conducted. For example, Sites and Crandall (1997) have cogently argued that, like all other propositions in science, species concepts need to be treated as hypotheses testable by strict and explicit criteria. Under the phylogenetic species concept, when two samples are distinct on the basis of at least one character state that is fixed in one and absent in the other, they become candidates for separate species (Davis and Nixon, 1992). However, there are several potential limitations to this approach. First, undersampling of attributes will reduce the discriminating power of the method and consistently bias the results toward the recognition of fewer species than actually exist. This factor necessitates the inclusion of as many relevant characters in a taxonomic analysis as is feasible. Second, if sample sizes of individuals are very small within a population, some attributes might appear fixed or absent from a population, when in fact they are polymorphic; undersampling of individuals thus biases the analysis toward the recognition of more species than might actually exist. One approach to avoiding the recognition of more species than actually exists is to incorporate principles of genealogical concordance (Avice and Ball, 1990) where concordant support from unlinked markers is used for diagnosis of species boundaries. This approach is particularly important when using mtDNA haplotypes to diagnose species because of the potential discordance between gene trees and species trees (Doyle, 1995).

Because of difficulties with previous analyses as previously cited, the unknown taxonomic status of Huachuca Mountain populations, and the advent of modern molecular techniques for addressing hypotheses of species status, a re-analysis of the

systematics of *Hyla eximia* and *H. wrightorum* is warranted. In this study, the evolutionary species concept is used as a theoretical framework for recognizing species (Frost and Kluge, 1995), and the phylogenetic species concept is used to operationally diagnose species and identify species boundaries (Davis and Nixon, 1992). Principles of genealogical concordance are used to corroborate species status, particularly when evaluating the phylogeny of cytochrome b haplotypes. We tested the hypothesis that *H. eximia* (sensu Duellman, 1970) is a single, widespread species distributed from Arizona to southern México against the alternative hypothesis that *H. eximia* and *H. wrightorum* are valid species.

In summary, the objectives of this project were to:

(A) Conduct field surveys to document the distribution and abundance of Huachuca mountain tree frog populations and other sensitive, threatened, and endangered amphibian and reptile species.

(B) Determine habitat quality where Huachuca tree frogs occur, including presence and abundance of non-native predators.

(C) Analyze geographic variation within *H. wrightorum* by comparing allozyme, cytochrome *b*, and advertisement call variation among populations from the Mogollon Rim, Huachuca Mountains, and the Sierra Madre Occidental of México.

(D) Evaluate the taxonomic status of *H. eximia* and *H. wrightorum* based on analyses of geographic variation.

METHODS

Field surveys and collections.--Exploration of the Huachuca and Canelo Hills area for assessment of known and unknown populations of the Huachuca tree frog and other sensitive amphibians and reptiles was conducted over a two year period (1996-1997). Fieldwork was conducted primarily during the summer monsoon season (July, August, and September) of each year when populations of Huachuca tree frogs tend to congregate at rain-formed pools and slow-moving streams to breed. Additional fieldwork was conducted during the month of June if appropriate weather conditions existed. Two primary survey methods were used. First, censusing of tanks, temporary pools, and slow-moving streams for breeding aggregations after summer rains allowed an assessment for presence or absence and number of reproductive individuals. Second, ponds and streams were censused for presence, abundance, and density of tadpoles when there was reason to believe tree frogs may have utilized a particular habitat for reproduction. This survey method allowed assessment for the presence of the Huachuca tree frog in areas that could not be immediately surveyed for adults after a rain storm. Whenever possible, a single adult specimen was collected or photographed as a voucher specimen from any locality discovered to have a breeding population. Latitude and longitude of surveyed localities were precisely documented in the field using a Garmin 12 XL Global Positioning System. Because Arizona tree frogs usually breed in shallow, ephemeral pools with subaquatic vegetation and emergent grasses, habitats with these characteristics were rated as "good" quality breeding sites. Ephemeral pools with little or no vegetation were rated

as “marginal” breeding sites, and relatively deep, permanent bodies of water were rated as “poor”. Habitat quality was rated independently from the presence or absence of non-native predators (e.g., crayfish, bullfrogs).

Male advertisement calls were recorded using a Marantz PMD 430 Stereo Recorder and Sennheiser ME 80 microphone. A minimum of 5 calls were recorded from each individual. Snout-vent length (SVL) of each recorded individual was measured to the nearest millimeter with a hand rule, and cloacal temperatures were measured with a Weber Quick Reading Thermometer immediately after the recording of each individual frog. Frogs were recorded from several areas representative of the geographic regions emphasized in this study including the Mogollon Rim, the Huachuclas, and the Sierra Madre Occidental (Figure 3).

Adults were collected and tissues taken from throughout the northern distribution of *Hyla eximia* (Figure 3) (Appendix 1). Along the Mogollon Rim, specimens were collected from Flagstaff (n = 16), East Clear Creek (n = 20), near McNary (n = 13), and north of Hannagan Meadow (n = 7). In the Huachucla Mountains, Huachucla tree frogs were collected from Turkey Creek (n = 6) and tributary to Scotia Canyon (n = 4). In México, Mountain tree frogs were taken from Sonora near the town of Yecora (n = 10). Sample size was often limited by the number of males observed calling; relatively small samples were taken from those populations with apparently small population densities. Liver and skeletal muscle were extracted and stored at -70 C. Liver representing three individuals collected from México D. F. were obtained from the Museum of Vertebrate Zoology at the University of California Berkeley (Appendix 1) for comparison with samples taken from the northern distribution of *Hyla eximia*.

Many other species of amphibians inhabiting the Huachuca mountains and Canelo Hills area are listed by the state of Arizona as Threatened or Endangered, including the Chiricahua leopard frog (*Rana chiricauihensis*), the barking frog (*Hylactophryne augusti*), and the Huachuca tiger salamander (*Ambystoma tigrinum stebbinsi*). Some species of amphibians and reptiles are candidates for listing, including the lowland leopard frog (*Rana yavapaiensis*), Mexican garter snake (*Thamnophis eques*), and ridge-nosed rattlesnake (*C. willardi willardi*). Still others are of special concern to the State of Arizona, and data are being actively accumulated and entered into computerized and manual files. These species include the Ramsey canyon leopard frog (*Rana subaquavocalis*), bunch grass lizard (*Sceloporus scalaris*), mountain skink (*Eumeces callicephalus*), banded rock rattlesnake (*Crotalus lepidus klauberi*), twin-spotted rattlesnake (*C. pricei*), western hooknose snake (*Gyalopion canum*), Mexican hognose snake (*Heterodon nasicus kennerlyi*), green rat snake (*Senticolis triaspis intermedia*), and Chihuahuan black-headed snake (*Tantilla wilcoxi*). Most of these species are found in or near the wet habitats where the Huachuca tree frog may be found. Observations of these species were noted during the course of fieldwork.

Starch gel electrophoresis procedures and analysis.--Liver and skeletal muscle were homogenized separately in a 1:1 (v:v) mixture of tissue and 0.01 M Tris-0.001 M EDTA-.001 M mercaptoethanol, pH 6.8. Homogenates were centrifuged at 12,000 g for 10 min. at 5 C. Within 72 h., the supernatant fractions were run on horizontal starch gels between approximately 0 and 5 C. Generally, supernatant fractions were used once and refrozen at -70 C in case further analysis was necessary. Standard horizontal starch gel

electrophoresis procedures were used (Murphy et al., 1990). Gels were composed of 12% hydrolyzed potato starch from Starch Art Corporation (Smithville, Texas). Enzyme stains follow Murphy et al. (1990). Locus homologies were estimated by relative staining intensities and mobilities in specific tissues. Enzymes, loci, tissue sources, and electrophoretic conditions are listed in Table 1. Electromorphs were labeled a, b, c, etc. in order of decreasing anodal mobility. Allelic designations are relevant to this study only.

Allozyme data were analyzed at several levels using Tools for Population Genetic Analysis (TFPGA), which was developed by M. Miller at Northern Arizona University. Descriptive statistics were calculated including allele and heterozygote frequencies, Nei's (1978) unbiased heterozygosity estimates, and percent polymorphic loci (based on percentage of loci that are not fixed for one allele). Next, genetic distances among sampled populations were calculated using Nei's (1978) unbiased genetic distance. Genetic distances were graphically represented using UPGMA, and bootstrap values were calculated for each node using 1000 replicates.

mtDNA Sequencing and Analysis.--DNA sequence data were collected from 30 individuals of *Hyla eximia* (3 from Flagstaff, 7 from Jone's Crossing, 5 from Hannagan Meadow, 6 from the Huachuca Mountains and Canelo Hills, 6 from Sonora, 3 from México D. F.) and five outgroup species (*H. arenicolor*, *H. cadaverina*, *H. cinerea*, *H. regilla*, and *H. squirella*). DNA was isolated from small amounts of liver (~100 mg) following the pheno/chloroform/isoamyl alcohol protocol of Hillis et al. (1996). The polymerase chain reaction (PCR) was used to amplify a 575 bp fragment of the

mitochondrial cytochrome b gene. The primers used to amplify this fragment were those used by Ptacek et al. (1994). Approximately 50-100 ng of total DNA was used as template in a standard double-stranded PCR amplification. PCR cycle parameters for this fragment were: 94°C for 30s, 50°C for 30s, and 72°C for 30s (40 cycles). Prior to sequencing the PCR products, unincorporated nucleotides and primers were removed using Wizard PCR Preps™ (Promega, Inc.). The purified DNA was dried and resuspended in sterile dH₂O. DNA templates were sequenced using a dye-labeled dideoxy terminator cycle sequencing kit (Applied Biosystems, Inc.) and an ABI 377 automated DNA sequencer (Applied Biosystems, Inc.). Sequences were analyzed and edited using the computer software program Sequencher™. The DNA sequences were aligned with Clustal W (Thompson et al., 1994). Because this fragment codes for a protein product, alignment was straightforward and unambiguous. Phylogenetic relationships among haplotypes were analyzed using the computer program PAUP, and sequences from *H. arenicolor*, *H. cadaverina*, *H. cinerea*, *H. regilla*, and *H. squirella* were used for outgroup comparison. Nodal support was tested through 200 replicates in a bootstrap analysis.

Call analysis.--In the laboratory, analog recordings of advertisement calls were digitized at a capture rate of 22,000 data points per second on a Macintosh PowerPC using Canary software (version 1.2.1). Call duration (CD) and pulse rate (PR) were measured in the waveform mode; CD was determined by measuring the total length of a call to the nearest 0.01 sec., and PR was calculated by dividing the total number of pulses per call by the call duration. Dominant frequency (DF) of advertisement calls was obtained from each

call using the spectrum mode of Canary with the following settings: frame length 16384 pts, time 2048 pts (87.5% overlap), FFT size 16384 pts, hamming filter, and amplitude quadratic to maximize frequency precision. Peak frequency was recorded as DF for every advertisement call. An average value for each call variable was obtained from no fewer than three calls, and these summary data were used for further analyses of call variation among Mogollon Rim, Huachuca, and Sonora populations.

Statistical analyses follow Sokal and Rolf (1981) and were conducted with SYSTAT 5.2 for the Macintosh (Evanston, IL, 1992). Because call variables typically vary significantly with body size and/or temperature, I first assessed the relationship of call variables to male SVL and to temperature. Variables significantly influenced by temperature were adjusted with the appropriate regression equation for analysis of size-related variation. To assess similarity among allopatric populations, ANOVA was used with Tukey pairwise comparisons.

RESULTS

Field Surveys-- Ephemeral aquatic habitats and cattle tanks received enough rainfall by late July, 1996, to fill basins with water and provide potential breeding habitat for Huachuca treefrogs. However, throughout the Huachuca there was very little rainfall during the summer of 1997, and none of the ephemeral aquatic habitats received enough rainfall to fill their basins. Hence, no breeding took place at the surveyed localities. The only locality observed to receive enough rainfall to allow breeding activity was in Turkey Creek (Table 2).

Sixteen separate springs, ponds, and stream reaches were surveyed for adults, tadpoles, or suitability of habitat for breeding or habitation by Huachuca tree frogs (Appendix I; Table 2). Virtually all of these sites consisted of artificial habitats (cattle tanks), but they were chosen because Huachuca tree frogs typically breed in rain-filled ponds or slow moving stretches of streams, and these localities fit this description. Although eleven of these localities were visited at night when male treefrogs potentially could have called, calling males were observed at only four sites (two separate sites along Turkey Creek, a tributary to Scotia Canyon, and Garden Canyon; Appendix I; Table 2). Tadpoles were observed in Turkey Creek, a tributary to Scotia Canyon, and Garden Canyon. Metamorphs were observed by Peter Holm in one of the Peterson ponds of Scotia Canyon (P. Holm, pers. comm.) in August 1996. Two juvenile treefrogs were collected by Mills Tandy (Fort Huachuca Wildlife Office) in Sawmill Canyon on Fort Huachuca. Eric Wallace (Arizona Game and Fish Department) heard three calling males in and near a small, ephemeral pond at the headwaters of Huachuca Canyon on 21 July 1998 at approximately 2000 hrs; approximately nine tadpoles were observed on 18 August 1998 (E. Wallace, personal communication). In all cases, Huachuca tree frogs and/or their tadpoles always were observed in temporary, rain-filled pools and were never associated with permanent bodies of water. The following includes general descriptions and accounts of surveyed habitats.

Canelo Hills Nature Conservancy Preserve (N 31° 33', W 110° 31').--Located in O'Donnell Canyon, relatively pristine cienaga habitat is present. Although several inches

of water covered much of the cienaga by August 1996 from summer rains, no Huachuca tree frogs or larvae were observed. Habitat good.

Turkey Creek (N 31° 33', W 110° 30').--Huachuca tree frogs previously were observed along Turkey Creek in the Canelo Hills. On 18 August 1997, a single tree frog was heard calling near the Ranger Station at approximately 2200 hrs. Twelve males were calling from a large, shallow, ephemeral pool upstream from the Ranger Station. The cienaga provided good breeding habitat, although the impacts of cattle in the creek and surrounding riparian areas is unknown.

Headwaters of Turkey Creek (N 31° 27', W 110° 27').--A relatively steep-sided, deep cattle tank was dry on 06 July 1996 but was full to approximately 20 x 15 meters on 16 July 1996; two Huachuca tree frogs were heard calling amongst the sparsely distributed emergent grasses at approximately 2330 hrs. No frogs or tadpoles were observed on 18 July and 15 September 1996 when this site was surveyed. Habitat marginal due to depth of tank, sparsity of aquatic and emergent vegetation.

Cattle Tank in Collins Canyon (N 31° 27', W 110° 29').--Located approximately 5 kilometers upstream from Parker Canyon Lake, this cattle tank was visited on 14 July 1996. The tank was full, but provided poor habitat for Huachuca tree frogs -- substrate was rocky and banks steep, and the tank was deep and virtually no aquatic or emergent vegetation was present. Several adult bullfrogs were observed, and crayfish were extremely abundant.

Neighbor Spring (N 31° 24', W 110° 28').--Located approximately two kilometers south of Parker Canyon Lake, the spring emerges from a steep hillside, and no potential breeding habitat was present. Because of the absence of any shallow basin for the collection of water, habitat was judged poor for Huachuca tree frogs.

Tributary To Upper Huachuca Canyon (N 31° 22', W 110° 24').--A shallow, ephemeral, artificial pond approximately 20 x 15 meters in size. This site remained dry throughout the summer of 1997 (E. Gergus, personal observation), but was reported to have water and appropriate vegetation for Huachuca tree frogs on 21 July 1998; three males were calling at approximately 2000 hours on this date (E. Wallace, personal communication). Approximately nine tadpoles were observed amongst the aquatic vegetation on 18 August 1998 (E. Wallace, personal communication). Breeding habitat marginal to good (further surveys required). A spring also was surveyed in Huachuca Canyon (N 31° 31', W 110° 23') during the summer of 1997; water levels were consistently low due to general absence of rain, but habitat was judged to be poor to marginal for Huachuca tree frogs because of the apparent absence of shallow basins or depressions for shallow pools of water to form for breeding habitat.

Upper Garden Canyon (N 31° 27', W 110° 22').-- By 18 July 1996, an artificial basin in upper Garden Canyon was full of water and emergent grasses were extremely dense creating a good habitat for breeding. Approximately ten males were calling, and two small egg masses were attached to emergent grasses just under the water's surface.

Crayfish were present within the breeding pond and other portions of the stream.

Sheridan Stone (Fort Huachuca Wildlife Biologist) heard frogs calling from this site at least one week previous to this date. Insufficient rains during the summer of 1997 left this basin virtually dry throughout the summer. A Black-tailed rattlesnake (*Crotalus mollosus*) was observed adjacent to the breeding site on 18 July 1996.

Carr Canyon, Comfort Spring (N 31° 25', W 110° 18').--The east side of the Huachuca Mountains is relatively steep and very little potential habitat is available for Huachuca tree frogs. This site was visited on 02 August 1997. The drainages in Carr Canyon have very steep gradients and rocky pools; no potential habitat was observed for Huachuca tree frogs.

Tributary to Scotia Canyon (N 31° 25', W 110° 22').--This site is characterized by a relatively large cattle tank (30 x 20 meters) of moderate depth within a creekbed tributary to Scotia Canyon. Immediately below the berm of the tank on the south side of the tributary lies a small, shallow basin that filled with water by 16 July 1996 when the associated cattle tank was also full. Approximately 18 Huachuca tree frogs called from the shallow basin which had a water depth of only a few inches and was largely filled and surrounded by emergent grasses. Three tree frogs called from the shallow margins of the cattle tank where emergent grasses were most dense. Four tree frogs were calling on 30 July, and tadpoles were observed in both the cattle tank and small pool below the berm. Approximately 12 tadpoles were observed on 15 September in 20 minutes of surveying margins of the ponds; several were near metamorphosis. The cattle tank seemed to

provide marginal to good habitat, and the small pool below the cattle tank was ranked as good.

On 13 June 1997, a small pool of water (1 x 2 meters) remained in the cattle tank. Approximately 50 juvenile bullfrogs were aggregated within the pool, and several were observed to take refuge within the mud cracks near the water. By 01 August, the tank was completely dry, but juvenile bullfrogs were living deep within the mud cracks.

Scotia Canyon, Peterson Ranch Ponds.--Five ponds are located within Scotia Canyon near the Peterson Ranch site. The lower pond (N 31° 27', W 110° 23') lies within the streambed of Scotia Canyon, is highly vegetated, relatively permanent, and has a relatively dense population of bullfrogs (Holm and Lowe, 1995; E. Gergus, personal observation). The middle pond (N 31° 27', W 110° 23') is highly silted, contains a dense stand of cattails (*Typha*), and seems to be a permanent body of water. The three upper ponds (N 31° 27', W 110° 23') are near the abandoned ranch; two of them are permanent bodies of water, the deeper of which consistently supports a population of bullfrogs and their tadpoles, and the eastern-most is a relatively shallow, ephemeral pond that has historically served as a breeding site for Huachuca tree frogs (Holm and Lowe, 1995). Although we did not observe tree frogs or tadpoles at this site during surveys fieldwork, Peter Holm (personal communication) did observe metamorphosing Huachuca tree frogs in August 1996. Natural history of Huachuca tree frogs in Scotia Canyon is discussed in Holm and Lowe (1995).

Sunnyside Canyon (N 31° 26', W 110° 23').--A portion of the creek flowing through Sunnyside Canyon just inside the Miller Peak Wilderness Area had shallow, marshy habitat with thick emergent grasses (15 September 1996); neither Huachuca tree frogs nor their tadpoles were observed, but the habitat appeared marginal to good for breeding activity. Further surveys should target this site when conditions are favorable for calling activity, or when tadpoles may be present.

Sycamore Spring (N 31° 23', W 110° 23').--This locality is characterized by a seepage within the creekbed which was visited only during 1997 when rainfall was minimal. No obvious depressions or basins were present which could fill with water and provide breeding habitat. This site should be surveyed following heavy monsoonal rains to survey for frogs or tadpoles. Overall, this habitat was ranked as marginal for Huachuca tree frogs. Two Sonoran Mud Turtles (*Kinosternon sonoriense*) were observed within a small, deep pool at the downstream end of the seepage.

Mud Tank (N 31° 23', W 110° 23').--This relatively small (8 x 5 meters), permanent tank was relatively deep, full of aquatic vegetation, and fed by a spring which was heavily grazed and trampled by cattle. Although the habitat seemed marginal for Huachuca tree frogs and was surveyed several times during 1997, particularly after small thunderstorms had occurred, no Huachuca tree frogs or their tadpoles were observed. This site should be surveyed following heavy thunderstorms, although the road leading to this site probably is rendered impassable after heavy rains. Approximately ten Sonoran mud turtles were consistently sited within this tank.

Lone Mountain Creek (N 31° 23', W 110° 21').--Although dry during 1997, several kilometers were surveyed to assess presence of any potential habitat (small basins that may fill with water), but none were observed. On the night of 18 August 1997, two *Hyla arenicolor* called from recently-filled pools approximately 50 meters upstream from the confluence with Bear Creek. The sighting of a Huachuca tree frog in 1993 (Holm and Lowe, 1995) indicates they may utilize some of these pools for breeding when conditions are appropriate. Habitat was marginal, but additional surveys should be conducted when conditions are appropriate.

Bear Spring (N 31° 24', W 110° 19').--This site had no apparent breeding habitat and was judged poor; steep slopes, narrow stream channel, and absence of pools or depressions precludes the presence of breeding Huachuca tree frogs. A Black-tailed rattlesnake was observed on 14 June 1997 near Bear Creek at approximately 2000 meters elevation.

Oversite Canyon (N 31° 22', W 110° 19').--Although this canyon is relatively steep and the creek channel rocky and narrow, a permanent spring is present, and several very small pools of water were observed within the creekbed. Horsetails (*Equisetum*) were common near the seepage. A Huachuca tree frog was sited within Oversite Canyon (Holm and Lowe, 1995), presumably near this locality. Besides the small pools within the creekbed, no breeding habitat was present; this site was judged poor to marginal.

Observations of other sensitive herpetofauna were limited during the course of fieldwork in 1996 and 1997. An adult Mexican garter snake (*Thamnophis eques*) was observed in the upper Peterson Ranch Ponds on 06 July 1996. An adult Banded rock rattlesnake (*Crotalus lepidus*) was observed on highway 83 approximately 12.5 miles south of Sonoita at 2215 hours. Although effort was expended during surveys to note incidental encounters with other species of special concern, no others were encountered during surveys.

Allozymes--. Twenty-one loci were resolved (Table 1) for all populations except México D. F., for which Ak, Ck, Fba, and Pk were not resolved because only liver tissue was available for analysis. Nine loci were monomorphic for all populations assayed (Aat-2, Ak, Ck, Ddh, Fba, Fumh, Iddh, Mdh-2, Pk) (Table 3). Although sample size was limited ($n = 3$), the México sample was fixed for a unique allozyme at 3 loci (Acon-2, Est-2, Sod), was polymorphic for a unique allozyme or allozymes at 2 loci (Aat-1 and Gpi), and was fixed for an allozyme shared with at least some other populations at 4 loci (Acon-1, Idh-2, Mpi, and Pgm). The Sonora population showed a unique allozyme at 3 polymorphic loci (Acon-1, Gpi, and Idh-2) and was fixed for an allozyme shared with at least some other populations at 2 loci (Idh-1 and Pgm). The Canelo and Huachuca populations shared identical character states and did not differ from Mogollon Rim populations in character state distributions except at the Mpi locus (Mpi^b for Huachuca and Canelo populations, Mpi^{ab} or Mpi^{abc} for Rim populations). Population genetic indices (average heterozygosities and percent polymorphic loci) were calculated for all populations (Table 4). Average heterozygosities within populations ranged from 0.1105

(Huachucas) to 0.1426 (McNary), and percent polymorphic loci ranged from 23.8 (Huachucas) to 33.3 (East Clear Creek and McNary). Nei's unbiased genetic distances ranged from 0.0020 (Flagstaff vs. East Clear Creek) to 0.6061 (Sonora vs. México D.F.) (Table 5). All populations exhibited a genetic distance of at least 0.4651 from the México D.F. population, but none of the former populations were separated by genetic distances greater than 0.0643 (Huachucas vs. Sonora) indicating relatively low levels of divergence among all populations except México D.F. An UPGMA phenogram, produced using Nei's (1978) unbiased genetic distances, illustrates the general similarity of all sampled populations with the exception of México, D.F. (Figure 6).

mtDNA--Percent divergence among Mountain treefrog samples ranged from 0.0-7.6% with the greater level of divergences appearing between the México D.F. samples and the remaining individuals. A total of nine different haplotypes were sampled (Figure 5). Two different haplotypes are found among the MVZ individuals, with these two haplotypes differing by only a single nucleotide substitution. The most frequent haplotype is "A" (observed in most Mogollon Rim individuals and one Sonoran sample), with haplotype "B" being derived from it (found in HM individual). The Sonoran individuals exhibit 3 different haplotypes (A,C, and F), and the Huachuca and Canelo Hills samples all exhibited the "G" haplotype. When analyzed with PAUP, a single most parsimonious phylogeny of the haplotypes was found (Figure 5). There were no convergent nucleotide changes; although some haplotype clades may be weakly supported, they are nonetheless diagnosed by unique nucleotide substitutions. Although some Sonoran haplotypes were more closely related to Rim haplotypes than to other

Sonoran haplotypes, all Huachuca samples exhibited the same unique haplotype (“G”), and these form a monophyletic haplotype clade. Similarly, although only three samples were available from México D.F., these also form a haplotype clade; additionally, this clade is supported by a large number of unambiguously unique nucleotides (14), while the Sonora-Huachuca-Mogollon Rim haplotype clade is supported by nine unambiguously unique nucleotides.

Advertisement calls.--For all populations as a group, temperature significantly influenced PR ($R^2 = 0.559$, intercept = 7.10, slope = 5.023, $F_{1,50} = 63.35$, $p < 0.001$; Figure 6) and CD ($R^2 = 0.478$, intercept = 0.415, slope = -0.011, $F_{1,50} = 45.71$, $p < 0.001$; Figure 7). Regression equations were calculated for each population and no significant relationship was found between CD and temperature; this was due to a short range of temperature variation among recorded individuals such that none of the recorded populations exhibited slopes that differed from zero. Since ANCOVA assumes homogeneity of slopes among samples when slopes are not equal to zero, ANCOVA was deemed unnecessary for further analysis. To test for differences in PR and CD among populations, ANOVA was performed by using their respective common regression equations to adjust PR and CD to a common temperature, 20°C; PR did not significantly vary among populations ($F_{2,49} = 2.11$, $p = 0.144$), but CD did ($F_{2,49} = 91.283$, $p < 0.001$) (Table 6). Tukey multiple comparisons indicated the Huachuca population was significantly different from both the Mogollon Rim and Sonora populations in CD, but the Mogollon Rim did not differ significantly from Sonora (Table 7).

For all populations as a group, SVL significantly influenced DF ($R^2 = 0.839$, intercept = 4.228, slope = -0.055, $F_{1,50} = 261.47$, $p < 0.001$; Figure 8). ANCOVA could not be employed because of heterogeneity of slopes; the regression equation from each population was used to adjust DF before using ANOVA to compare populations. DF significantly varied among populations ($F_{2,49} = 179.61$, $p < 0.001$) (Table 6), and Tukey comparisons indicated significant differences among all three populations (Table 7). Size (SVL) also varied among populations (ANOVA, $F_{2,49} = 74.576$, $p < 0.001$) (Table 6); Tukey pairwise comparisons indicated Huachuca frogs were significantly smaller than Mogollon Rim frogs, and Sonora frogs were smaller still (Table 7).

DISCUSSION

Natural history and demography.--The success of field surveys was greatly affected by rainfall amount during the summer months of 1996 and 1997. The potential habitats which were surveyed during the 1996 field season all received sufficient rainfall to fill cattle tanks or other bodies of water that provided potential breeding sites for Huachuca tree frogs. For localities where Huachuca tree frogs were observed, the first siting at any given locality followed soon after the given body of water was formed and emergent vegetation was present. Thus, calling activity was usually observed one or two days following a storm event in which large, ephemeral pools were formed. Earlier summer rainfall tended to stimulate the growth of grasses within an earthen basin or cattle tank, even before standing water persisted, thereby providing refuge for calling males. Huachuca tree frogs tended to call from grasses or other low-lying vegetation while floating on the water's surface or just outside of the water on the substrate. Choruses of

ten to twenty frogs were heard within 50 meters of the calling site; one could identify that a chorus was present if within close proximity of a breeding aggregation, but beyond this approximate range calling generally could not be heard. Thus, one generally must be within relatively close proximity of calling males if they are to be detected. Calling activity often would temporarily subside when quietly approached, but would initiate again when disturbances were minimal. In most instances, we arrived at chorus sites well after dark and chorusing was already underway. However, the tank located in the tributary to Scotia Canyon (Figure 5) was attended before dark on 16 July 1996. Some frogs called sporadically beginning around 1930 hrs, and chorusing activity was relatively constant by 2045 hrs. Similar observations were made in Sonora, México on 18 July 1997, where a relatively large population was encountered; males began calling at approximately 1745 hours, and several hundred were chorusing by approximately 2100 hrs. Although males may occasionally call during daylight hours, most calling activity probably occurs after twilight, and surveys targeting these frogs should concentrate field efforts accordingly.

Populations of Huachuca treefrogs may be susceptible to local extirpation by alteration of habitats and predation by non-native predators. Mountain treefrogs seem to require relatively small, shallow, ephemeral pools for chorusing, mating, and larval development. Indeed, all frogs and larvae encountered in this study were observed in such habitats, and other populations exhibit similar patterns (Duellman, 1970). Topography of the Huachuca Mountains is relatively steep (Bowers and McLaughlin, 1996) and potential breeding sites seem relatively scarce. Among surveyed sites where Huachuca treefrogs were not found, all except one lacked habitat characteristics associated with

typical breeding habitat; portions of the creek draining Sunnyside Canyon within the boundaries of the Miller Peak Wilderness had relatively low gradient stretches with marsh like habitats that seemed appropriate for habitation by adults and larvae. Other surveyed sites were typified either by relatively high gradient streams with rocky substrates or relatively deep cattle tanks. Because of the rarity of appropriate breeding habitats in the Huachuclas, severe alteration or disturbance of any or all of these could have severe impacts upon the persistence of viable Huachuca treefrog populations in the region. In summary, Huachuca treefrogs probably breed relatively regularly in Lower Turkey Creek, the tank located in the tributary to Scotia Canyon and its associated marshy pond below its dam, a single pond in Scotia Canyon at the historic Peterson Ranch site, upper Garden Canyon, and a tributary to upper Huachuca Canyon. For conservation and management purposes, it would be fruitful to identify other regular breeding sites, although at this time such locations are unknown.

Several cattle tanks had permanent water and abundant aquatic and subaquatic vegetation (e.g., Peterson Ranch ponds, Mud Springs), however, neither frogs nor tadpoles were observed at these localities. Huachuca treefrogs may avoid these sites because of natural predators, such as various insect larvae and small fishes, that may be associated with them. Non-native predators, such as crayfish (*Orconectes*) and bullfrogs (*Rana catesbeiana*), were found at some of these localities and may account for the absence of Huachuca treefrogs from them; bullfrogs are known to prey upon small vertebrates and are possible causes of the extirpation or extinction of several species of ranid frogs (Hayes and Jennings, 1986). For example, lower and upper Peterson Ranch ponds have permanent water, bullfrogs were present upon every visit, and neither

treefrogs nor tadpoles were observed in them; these results are consistent with those of Holm and Lowe (1995) who only observed breeding activity of Huachuca treefrogs in one of the ponds. Two adult bullfrogs were seen along the margins of tank within the tributary to Scotia Canyon in July 1996 where Huachuca treefrogs were observed to breed. In June 1997, this tank was observed to be nearly dry when at least 50 juvenile bullfrogs were observed to be crowded within the remaining small pool of water. Later in the summer the tank was completely dry, but several bullfrogs were observed taking refuge deep within the cracked mud in the tank's basin. Clearly, bullfrogs are able to endure local drying events to some degree, and thus may not be excluded from habitats with ephemeral bodies of water. However, given that bullfrog tadpoles typically metamorphose after a year or longer (Viparina and Just, 1975), successful breeding may be rare to absent in many of the ephemeral habitats utilized by Huachuca tree frogs. Bullfrogs appeared to be absent from other bodies of permanent water, such as Mud Tank, where numerous Sonoran Mud Turtles (*Kinosternon sonoriense*) were consistently observed during the course of this study. Sonoran mud turtles are native to the region, but it is unknown whether they prey upon larvae of Huachuca tree frogs. Although bullfrogs are thought to negatively impact local populations of leopard frogs, including *Rana yavapaiensis* and *R. chiricauhensis*, impact on Huachuca treefrogs has not been tested. Given that treefrogs tend to disperse shortly after metamorphosis, they may be most vulnerable to predation by bullfrogs following metamorphosis and again when mature individuals return to ponds for breeding activities. Collections of bullfrogs during these events with subsequent gut-content analysis may reveal whether Huachuca tree frogs are preyed upon at these times.

Introduced crayfish (*Orchonectes*), which are known to heavily graze local habitats and have negative impacts on native aquatic herpetofauna in Arizona (Fernandez and Rosen, 1996), were abundant in the Upper Garden Canyon site and are known to occur in other drainages of the Huachucas such as Bear Creek on the western slopes (Holm and Lowe, 1995; E. Gergus, personal observation). It is not known what impact, if any, crayfish may have on Huachuca treefrogs and their eggs or larvae.

Geographic Variation And Its Significance.--Allozyme evidence indicates low levels of differentiation among populations of the Mogollon Rim (genetic distances ranged from 0.0020 to 0.0287) and between the Huachucas and Mogollon Rim populations (maximum genetic distance was 0.0221). The only qualitative difference between Mogollon Rim and Huachuca populations are found at the Idh-1 and Mpi loci where the Huachuca sample was monomorphic at each locus, whereas the Mogollon Rim populations were polymorphic. However, a greater level of differentiation exists between both Arizona samples (Mogollon Rim and Huachuca populations) and the Sonora population (genetic distance 0.0643 between Huachucas and Sonora, and at least 0.0571 between Mogollon Rim and Sonora). The Sonora sample showed qualitative differences between the Huachucas and Mogollon Rim with several allozyme morphs being found only in the Yecora sample, although in polymorphic states (Table 3). The México D.F. sample was highly divergent from all others (minimum genetic distance 0.4651) with several fixed qualitative differences (Table 3). For instance, Aat-1^c was fixed among all northern populations (total sample size = 76), whereas the México D.F. sample uniquely exhibited the Aat-1^a and Aat-1^b morphs. Similarly, all northern populations were monomorphic at

the Acon-2, Est-2, and Sod loci while the México D.F. sample was fixed for alternative allozymes.

Maximum parsimony analysis of cytochrome b variation indicates that haplotypes of some individuals sampled from the Mogollon Rim are more closely related to haplotypes of Sonoran frogs than other Mogollon Rim frogs (Figure 7). For example, the “F” haplotype from Sonora is more closely to the “D” and “E” haplotypes of Hanagan Meadow frogs than to other Sonoran frogs (haplotypes “C” and “A”). In fact, most of the frogs sampled from the Mogollon Rim exhibited the “A” haplotype, while a single Sonoran frog also possessed the same haplotype. Huachuca tree frogs possessing haplotype “G” form a monophyletic group that is the sister clade to all Sonoran and Mogollon Rim samples, and the three samples from southern México (México D.F.) form a haplotype clade which is sister to all other samples of Mountain tree frogs. Not only does the “G” haplotype represent unique evolution within the Huachuca population, but the apparent exclusivity of this population suggests the Huachuca populations have been evolving independently from other Mountain tree frog populations. Small population sizes in the Huachuca region may explain, in part, rapid coalescence of this haplotype. Straight line distance between Yecora and México D.F. is approximately 1375 km, whereas the distance between Yecora and Flagstaff is approximately 805 km. Sonora and Mogollon Rim haplotypes show relatively low levels of divergence (a maximum of 8 nucleotide differences between haplotype “F” in Sonora and haplotypes “A” and “B” along the Mogollon Rim), and an equivalent level of divergence was observed among the Mogollon Rim samples alone. However, the minimum level of nucleotide differentiation between Yecora and México D.F. is 26 nucleotide substitutions between haplotype “H”

of México D.F. and haplotype “F” of Sonora. In summary, phylogenetic relationships among cytochrome b haplotypes indicates the nonexclusivity of Sonora and Mogollon Rim tree frogs, while the exclusive nature of the Huachuca “G” haplotype indicates this population is evolving independently from all others. Similarly, the sample from México D.F. indicates that these populations also may be evolving independently from those in northern México and Arizona, although lack of sampling from intervening sites may account for this result; the apparent exclusivity of México D.F. from other sampled populations may be artifactual.

Comparisons of advertisement components revealed statistically significant differences in SVL adjusted DF and temperature adjusted CD among all populations. Frogs from the Huachucas had DFs nearly 200 Hz higher than those of the Mogollon Rim, and Sonoran frogs were about 100 Hz higher than those from the Huachucas. However, there were no significant differences among populations in temperature adjusted PR (Table 6). In fact, the Sonora and Huachuca samples exhibited nearly identical PRs, while the Mogollon Rim population was slightly lower. Despite the fact that the differences in DF and CD among these populations were statistically significant, the biological significance of these differences is questionable. Pulse rate is perhaps the most important call component in mate recognition (Gerhardt, 1988), yet PR has not diverged among populations sampled in this study; based upon PR alone, females from any particular population would probably identify a male from any other population as a potential mate. Despite the fact that DF and CD have diverged among these populations, differences are probably not biologically significant (Gerhardt, 1988) but do imply that these allopatric populations may have differentiated to a relatively minor extent.

Significant differences in SVL also suggest independent evolution among these allopatric entities (Table 6); these results are concordant with those of others (Duellman, 1970; Renaud, 1977; Taylor, 1938). Both Renaud (1976) and Taylor (1938) used differences in SVL, in part, to argue for the recognition of *H. eximia* and *H. wrightorum*. Historically, however, size differences may have varied in a geographically continuous manner and present patterns may merely reflect historically clinal variation.

Mountain tree frogs appear to be continuously distributed throughout their distribution in mainland México, thereby providing opportunity for gene flow between the most northern and southern populations if a single species is represented by these populations. Given that discrete differentiation of allozymes and mtDNA was detected between northern (Sonora) and southern (México D.F.) populations (Table 3, Figure 7), these results might support the hypothesis that at least two independently evolving lineages (species) are represented by the data. Given the limited geographic sampling in this study, however, such a conclusion is only weakly supported; allozyme and mtDNA may vary clinally, and isolation-by-distance may exist. These alternatives can be tested with further sampling in mainland México. Based upon significant differences in pulse rate, Blair (1960) distinguished “Fast *eximia*” from “Slow *eximia*” in southern México. Although cloacal temperatures were not reported, both air and water temperatures were. Because Mountain treefrogs usually call while partially submerged in water, water temperature probably provides a relatively close approximation of cloacal temperature; “Slow *eximia*” were recorded between 21 and 23°C and had pulse rates between 41 and 53 p/s, while “Fast *eximia*” and *Hyla wrightorum* from Arizona were recorded between 19.0 and 22.0°C had pulse rates between 100 and 136 p/s. At roughly comparable water

temperatures, PRs of “Slow *eximia*” are roughly half those of “Fast *eximia*” and *H. wrightorum*; because this magnitude of difference is common for pairs of sympatric species (Gerhardt, 1988), Blair’s (1960) results are very suggestive of cryptic species within *Hyla eximia* of México and may help to explain the discrete allozyme and mtDNA differences between Sonora and México D.F. samples observed in this study.

Taxonomic Implications.--As far as taxonomy of Mountain treefrogs in Arizona relative to mainland México, data are suggestive of multiple, independently evolving lineages, but strong, unambiguous evidence (e.g., fixed character states) is lacking. Data from allozymes, advertisement calls, mtDNA sequences, and/or morphology cannot be used to unambiguously reject the hypothesis that Mogollon Rim, Huachuca, and Sierra Madre Occidental Mountain treefrogs represent a single species. Some of the data suggest clinal variation: DFs in advertisement calls increase gradually from north to south (Table 6), and SVL decreases from north to south (Table 6). Although populations of the Mogollon Rim, Huachucas, and mainland México appear to be geographically isolated, these phenotypic patterns may reflect clinal patterns of variation which existed when these populations were continuously distributed. Alternatively, if these differences have evolved in situ, phenotypic differences are not discrete in nature but character distributions overlap among geographic entities. Under a conservative application of the ESC and under the Biological Species Concept (BSC), these allopatric entities should be regarded as conspecific, despite the fact that current geographic distribution suggests that these represent independently evolving lineages.

The phylogeny of the cytochrome b haplotype suggests fixation of the unique “G” haplotype in the Huachuca and Canelo Hills populations (Figure 7), a pattern suggestive of the Huachuca populations evolving independently from all others. However, the fixation of diagnosable mtDNA haplotypes is an expected outcome in small populations because the small inbreeding effective population size (N_e) for nonrecombining haploid loci means that they will sort to fixation about four times faster than a single-copy Mendelian locus (Birky et al., 1989). The use of mtDNA markers alone, therefore, will identify population units that are demographically independent over ecological time (Moritz 1994, 1995; Avise 1995), but diagnosis of such groups as species in the absence of support from independent characters would trivialize the concept of species as evolutionarily independent lineages (Kluge, 1990). Concordant support from independent markers would conservatively identify the Huachuca populations as a diagnosable species -- concordances are likely to arise only when populations have been separated from one another for long periods of time (i.e., they are independently evolving lineages) -- such markers are lacking in this study. Likewise, because of no discrete character state differences between Mogollon Rim populations and those in the Huachuca Mountains and Sonora, México, the Mogollon Rim populations cannot be unambiguously diagnosed from those to the immediate south; these entities collectively represent a single species comprised of allopatric geographic variants. The Huachuca populations can be recognized by their haplotype divergence, which represents phylogeographic diversity which may be recognized for its own conservation value (Allendorf and Leary, 1988; Bowen et al., 1994). Using the terms of Moritz (1994, 1995), Huachuca tree frogs represent *management units* -- a set of the total population that is currently

demographically isolated -- and to a lesser extent an *evolutionarily significant* unit -- a historically isolated population that represents a component of evolutionary diversity within a taxon. Indeed, the geographically restricted nature of these populations, coupled with their minimal but existing divergence from other populations, suggests some level of protection should be accorded to them.

However, a diagnosable entity does appear to exist in southern México, as is evidenced from allozymes, mtDNA, and advertisement calls, that has significantly diverged from populations to the north and represents an independently evolving lineage that should be recognized as a separate species. Names currently exist for these species - - the southern form is represented by *Hyla eximia*, and the form to the north is *Hyla wrightorum*. Although molecular character states my clinally vary such that these are not, in reality, independently evolving lineages, evidence from advertisement calls is not consistent with this interpretation; the PR of *Hyla wrightorum* is nearly double that of *H. eximia*, a difference consistent with most other North American hylids which exhibit intrinsic reproductive isolating barriers (Gerhardt, 1988). Although further field and laboratory research should be done to identify the geographic area of contact between *H. eximia* and *H. wrightorum*, current evidence is sufficient to at least formally recognize them as distinct species, regardless of whether the ESC or BSC is applied.

Summary.--Field surveys of the Huachuca Mountains and Canelo Hills in southeastern Arizona for Huachuca tree frogs (*Hyla wrightorum*) during the summers of 1996 and 1997 indicated that breeding populations are localized and relatively small. During 1996, frogs were observed in Turkey Creek, in a tributary to Scotia Canyon, in Garden Canyon,

and in one of the Peterson Ranch ponds. Chorus densities were small with 12 males observed in lower Turkey Creek, two males in upper Turkey Creek, approximately 21 in the tributary to Scotia Canyon, and ten in upper Garden Canyon. Tadpoles and metamorphs were observed at the Peterson Ranch site. During 1997, rainfall was scarce, and frogs only were observed in Turkey Creek ($n = 12$). During 1998, frogs were reported from an additional site in upper Huachuca Canyon. All frogs were found in habitats typical for *Hyla wrightorum*; frogs and tadpoles were absent from other surveyed sites, most of which had poor to marginal habitats for Huachuca tree frogs. Non-native predators, such as crayfish and bullfrogs, were observed at some sites, but their impacts on Huachuca tree frogs remain unknown. Analyses of allozyme, mtDNA, and advertisement call variation suggest Mogollon Rim, Huachuca, and Sonoran populations have differentiated very little from one another; lack of discrete diagnostic character state differences (with the exception of Huachuca tree frog haplotype divergence) precludes their recognition as separate species. However, analysis of a small sample from southern México provides evidence that two species are present with *Hyla eximia* representing the southern form and *H. wrightorum* the northern form. Due to the localized nature of habitat suitable to Huachuca tree frogs and their apparent small population sizes, measures should be taken to prevent habitat alterations, or even to improve current habitats. Further field surveys should be periodically conducted in the Huachuca Mountains and Canelo Hills to track population trends of Huachuca tree frogs so that reliable base-line data will be available for more sound conservation and management decisions.

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TABLE 1.--Enzymes, loci, International Union of Biochemistry Nomenclature Committee numbers, tissue sources, and buffer systems.

Enzyme	IUBNC No.	Locus	Tissue source	Buffer system*
Aconitase hydratase	4.2.1.3	Acoh-1	Liver	A
		Acoh-2	Liver	A
Adenylate kinase	2.7.4.3	Ak	Liver	A
Aspartate aminotransferase	2.6.1.1	Aat-1	Liver	B
		Aat-2	Liver	A
Creatine kinase	2.7.3.2	Ck	Muscle	A
Dihydrolipoamide dehydrogenase	1.8.1.4	Ddh	Liver	C
Esterase (nonspecific)	3.1.1.-	Est-2	Liver	B
Fructose-bisphosphate aldolase	4.1.2.13	Fba	Muscle	A
Fumarate Hydratase	4.2.1.2	Fumh	Muscle	A
Glucose-6-phosphate isomerase	5.3.1.9	Gpi	Liver	A
Glycerol-3-phosphate dehydrogenase	1.1.1.8	G3pdh	Muscle	B
L-Iditol dehydrogenase	1.1.1.14	Iddh	Liver	C
Isocitrate dehydrogenase	1.1.1.42	Idh-1	Liver	B
		Idh-2	Liver	B
Malate dehydrogenase	1.1.1.37	Mdh-1	Liver	B
		Mdh-2	Liver	B
Mannosephosphate isomerase	5.3.1.8	Mpi	Liver	B
Phosphoglucomutase	5.4.2.2	Pgm	Liver	B
Pyruvate kinase	2.7.1.40	Pk	Muscle	B
Superoxidase dismutase	1.15.1.1	Sod	Liver	C

*A = Amine-citrate (morpholine), pH 6.1; B = Tris-citrate II, pH 8.0; C = Tris-borate-EDTA I, pH 8.6

TABLE 2.--Brief summary of field surveys for Huachuca tree frogs in the Huachuca mountains and Canelo Hills region. See text for descriptions of sites visited and specific locality information.

Date	Locality	Observations
05 July '96	Peterson Ranch site	2045-2130 hrs. Small pool of water present in ephemeral pond. One bullfrog and one Mexican garter snake sighted.
06 July	Upper Turkey Creek	Dry.
	Lower Turkey Creek	Dry.
16 July	Trib. to Scotia Canyon	Pond basin with some water (10 m x 12 m).
	Tributary Scotia Canyon	Arrived at 1845 hrs. Pond basin full. Tree frogs began calling at 1930 hrs, full chorus by 2045 hrs. Chorus size estimated at 25 individuals; one pair amplexed.
17 July	Lower Turkey Creek	Dry.
	Peterson Ranch site	2100 hrs. Walked around pond perimeter and listened for calling activity; tree frogs absent.
18 July	Upper Garden Canyon	2000-2200 hrs. Pond full. Approximately 10 tree frogs observed calling. Two egg clutches observed.
30 July	Tributary Scotia Canyon	2045-2130 hrs. 4 tree frogs calling, 1 female observed. ~30 <i>Hyla</i> tadpoles observed in 15 minutes.
14 Sept.	Lower Turkey Creek	Dry.
	Upper Turkey Creek	Surveyed margins of tank after dark for adults and tadpoles; none observed.
15 Sept.	Tributary Scotia Canyon	Observed several <i>Hyla</i> tadpoles metamorphosing; ~12 with hindlimbs well developed.
13 June '97	Mud Spring	About 8 <i>Kinosternon sonoriense</i> observed in pond.
	Sycamore Spring	Several pools of water in creekbed; large <i>K. sonoriense</i> observed.
	Tributary Scotia Canyon	Small pool of water remaining (~2 m x 3 m); ~50 juvenile bullfrogs observed in pool, some in nearby mud cracks.
	Neighbor Spring	Seepage on steep slope; no breeding habitat for frogs. Spring located on steep slope; no potential breeding habitat.
14 June	Bear Spring	Small seepage; no breeding habitat for tree frogs.
	Upper Garden Canyon	Creekbed dry.
14 July	Tank in Collins Canyon	Large, steep-sided tank; crayfish abundant, 3 adult bullfrogs observed.
15 July	Mud Spring	~10 <i>K. sonoriense</i> . Spring heavily grazed and trampled.
	Lone Mountain Creek	Creekbed with rocky substrate; no apparent Mountain tree frog habitat.
	Oversite Canyon	Steep gradient slope with 40-meter long spring; <i>Equisetum</i> abundant; few very small pools. Mountain tree frog habitat marginal.
	Upper Huachuca Canyon	Hiked up stream to top of ridge; very steep, some small

01 Aug.	Lower Turkey Creek	pools, marginal habitat.
	Upper Turkey Creek	Dry.
	Tributary Scotia Canyon	Small pool of water; insufficient for breeding.
	Peterson Ranch site	Pond dry, bullfrogs taking refuge in mud cracks.
	Mud Spring	Dry.
		Recent rain evident. Arrived after dark; no tree frogs or tadpoles observed.
	Sycamore Spring	2200 hrs. Recent rain evident. No tree frogs or tadpoles.
02 Aug.	Oversite Canyon	No change since 15 July; creekbed dry, spring with small pools.
	Carr Canyon	Steep slopes; no habitat for Huachuca tree frogs
18 Aug.	Upper Garden Canyon	Dry.
	Canelo Preserve	Cienaga marshy, but insufficient water for breeding of Huachuca tree frogs; none observed.
	Lower Turkey Creek	Water flowing in creekbed. ~12 Huachuca tree frogs called between 2200 and 0000 hrs.
	Tributary Scotia Canyon	Dry.
19 Aug.	Mud Spring	2100 hrs. Searched pond margins; no tree frogs.
	Peterson Ranch site	Basin filled with 6 m x 6 m water, but no emergent grasses. No tadpoles or frogs encountered.
12 Sept.	Upper Garden Canyon	Breeding site with small pool; insufficient for breeding
	Tributary Scotia Canyon	Dry.
	Peterson Ranch site	Dry.
	Mud Spring	Tank still full; <i>K. sonoriense</i> observed.
	Upper Garden Canyon	Dry.
	Lower Turkey Creek	~10 Huachuca tree frog tadpoles observed.

TABLE 3.--Distribution of genotypes at 21 loci for sampled populations of tree frogs in Arizona and México. Migration distance is given in millimeters, and relative mobilities are designated by letters (e.g., a, b, c, etc.).

Locality Locus	Flagstaff	East Clear Creek	McNary	Hannagan Meadow	Huachucas/Canelo	Sonora	Mexico, D.F.
Aat-1	6 c	6 c	6 c	6 c	6 c	6 c	14/9 ab
Aat-2 (cath)	23 a	23 a	23 a	23 a	23 a	23 a	23 a
Acon-1	36/33 bc	36/33 bc	36/33 bc	36/33 bc	36/33 bc	39/36/33 abc	33 c
Acon-2	6 a	6 a	6 a	6 a	6 a	6 a	7 (cath) b
Ak	25 a	25 a	25 a	25 a	25 a	25 a	NA
Ck	18 a	18 a	18 a	18 a	18 a	18 a	NA
Ddh	30 a	30 a	30 a	30 a	30 a	30 a	30 a
Est-2	50 b	50 b	50 b	50 b	50 b	50 b	60 a
Fba	14 a	14 a	14 a	14 a	14 a	14 a	NA
Fumh	15 a	15 a	15 a	15 a	15 a	15 a	15 a
G3pdh	35/29 ab	35/29 ab	35/29 ab	35/29 ab	35/29 ab	35/29 ab	35/29 ab
Gpi	13/20 cd	13/20 cd	20/13 cd	20/13 cd	13/20 cd	13/20/24 bcd	20/28 ac
Iddh	26 a	26 a	26 a	26 a	26 a	26 a	26 a

Idh-1	56/48 ab	56/48 ab	56/48 ab	56/48 ab	56 a	56 a	56 a
Idh-2	15 b	15 b	15 b	15 b	15 b	27/15 ab	15 b

TABLE 3.--Continued.

Locality Locus	Flagstaff	East Clear Creek	McNary	Hannagan Meadow	Huachucas/ Canelo	Sonora	Mexico, D.F.
Mdh-1	30/22 ab	30/22 ab	30/22 ab	30/22 ab	30/22 ab	30/22 ab	22 ab
Mpi	62/58/53 abc	62/58/53 abc	62/58/53 abc	62/58 ab	58 b	62/58/53 abc	53 c
Pgm	46/42/38 abc	46/42/38 abc	46/42 ab	46/42 ab	46/42 ab	42 b	38 c
Pk	9 a	9 a	9 a	9 a	9 a	9 a	NA
Sod	53 a	53 a	53 a	53 a	53 a	53 a	45 b

TABLE 4.-- Average heterozygosities and percent polymorphic loci for populations represented by samples of 10 or more individuals.

Locality	Average Heterozygosity	% Polymorphic Loci
Flagstaff	0.1211	28.6
Jone's Crossing	0.1325	33.3
McNary	0.1426	33.3
Huachucas	0.1105	23.8
Sonora	0.1190	28.6

TABLE 5.--Matrix of Nei's (1978) unbiased genetic distances (above diagonal) and identities (below diagonal) of treefrog populations. Population codes are as follows: 1 = East Clear Creek, 2 = Flagstaff, 3 = McNary, 4 = Hannagan Meadow, 5 = Huachucas, 6 = Sonora, 7 = México D.F.

	1	2	3	4	5	6	7
1	-----	0.0020	0.0072	0.0240	0.0165	0.0592	0.5067
2	0.9980	-----	0.0165	0.0287	0.0221	0.0704	0.4651
3	0.9928	0.9837	-----	0.0074	0.0085	0.0583	0.5237
4	0.9763	0.9717	0.9926	-----	0.0154	0.0571	0.5274
5	0.8836	0.9781	0.9916	0.9848	-----	0.0643	0.5590
6	0.9425	0.9320	0.9434	0.9445	0.9377	-----	0.6061
7	0.6025	0.6281	0.5923	0.5901	0.5718	0.5455	-----

TABLE 6.--Temperature adjusted (20°C) advertisement call variables (PR and CD), SVL adjusted DF, and SVL for allopatric populations of Mountain tree frogs. Values are mean \pm SE.

Population	PR (p/s)	CD (s)	DF (kHz)	SVL (mm)	n
Mogollon Rim	104.3 \pm 1.82	0.240 \pm 0.006	2.007 \pm 0.013	41.1 \pm 0.49	23
Huachucas	110.0 \pm 2.75	0.119 \pm 0.007	2.190 \pm 0.037	35.3 \pm 0.69	15
Sonora	110.3 \pm 3.36	0.223 \pm 0.007	2.291 \pm 0.017	31.4 \pm 0.60	14

TABLE 7.-- Pairwise probability matrices for Tukey multiple comparisons among Mogollon Rim, Huachuca, and Sonora populations of Mountain tree frogs. PRs and DFs are above the diagonal of the first and second panels, respectively. CDs and SVLs are below the diagonals of the first and second panels, respectively.

	Mogollon Rim	Huachucas	Sonora
Mogollon Rim	-----	0.231	0.221
Huachucas	0.014	-----	0.998
Sonora	0.000	0.000	-----
Mogollon Rim	-----	0.000	0.212
Huachucas	0.000	-----	0.000
Sonora	0.000	0.000	-----

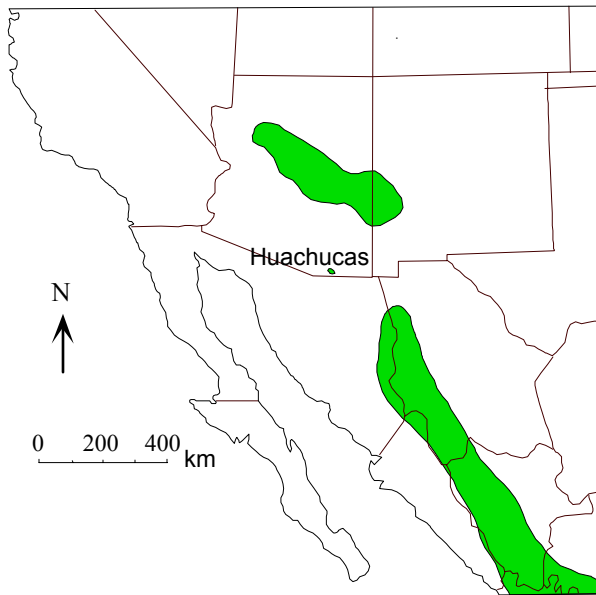


FIG 1.--Distribution of Arizona tree frogs (*Hyla wrightorum*) and Mountain tree frogs (*H. eximia*). According to Taylor's (1938) original description of *H. wrightorum*, its distribution includes Arizona, New Mexico, and northern México, although the contact zone with the more southern *H. eximia*, which extends south to Guatamala, was ambiguous. See text for details on distribution and taxonomic history of these two taxa.

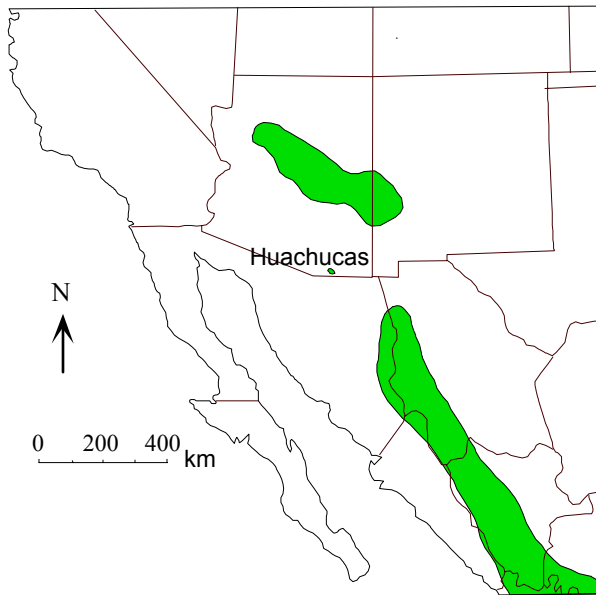


FIG 1.--Distribution of Arizona tree frogs (*Hyla wrightorum*) and Mountain tree frogs (*H. eximia*). According to Taylor's (1938) original description of *H. wrightorum*, its distribution includes Arizona, New Mexico, and northern México, although the contact zone with the more southern *H. eximia*, which extends south to Guatamala, was ambiguous. See text for details on distribution and taxonomic history of these two taxa.

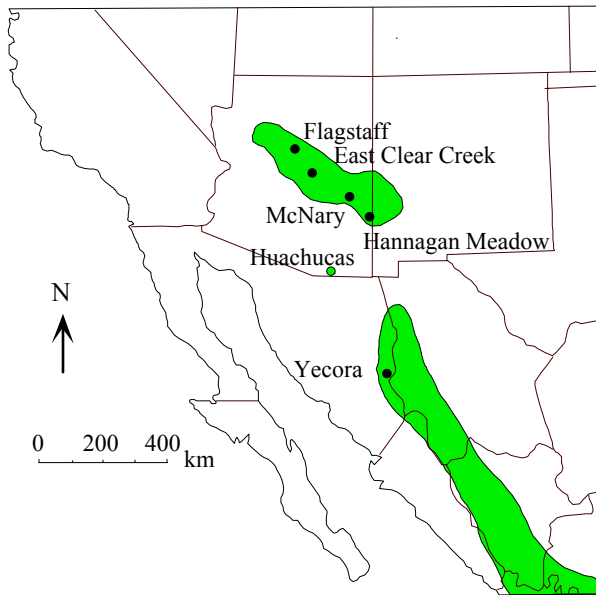


FIG. 3.--Sample localities for the collection of tissue samples and recording of advertisement calls. See text for exact localities.

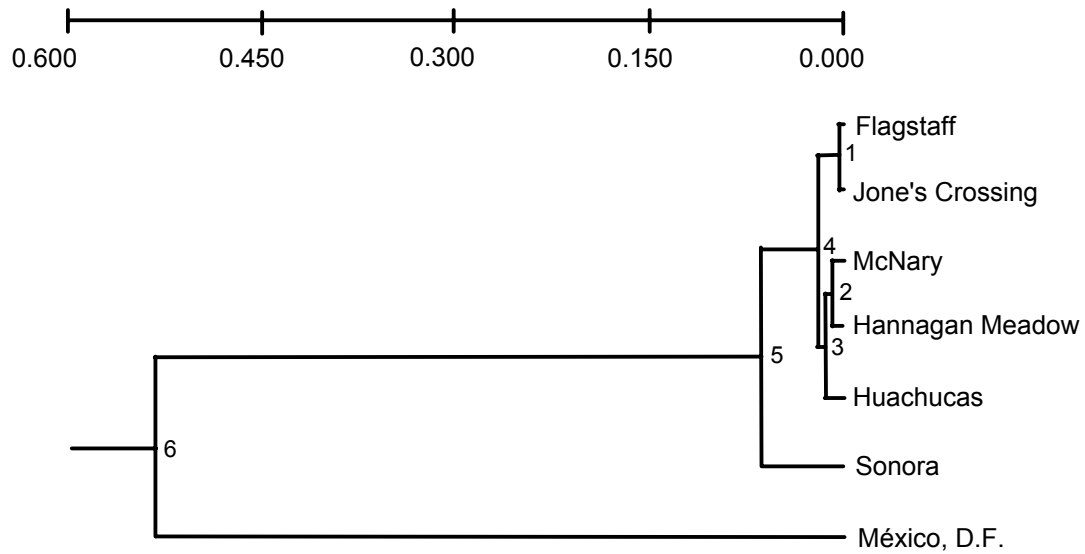


FIG. 4.--UPGMA phenogram of Nei's (1978) unbiased genetic distances of mountain treefrog populations. Nodal support from bootstrap analysis (1000 replicates) is as follows: Node 1: 0.6540; Node 2: 0.3940; Node 3: 0.2970; Node 4: 0.0192; Node 5: 0.9980; Node 6: 1.000.

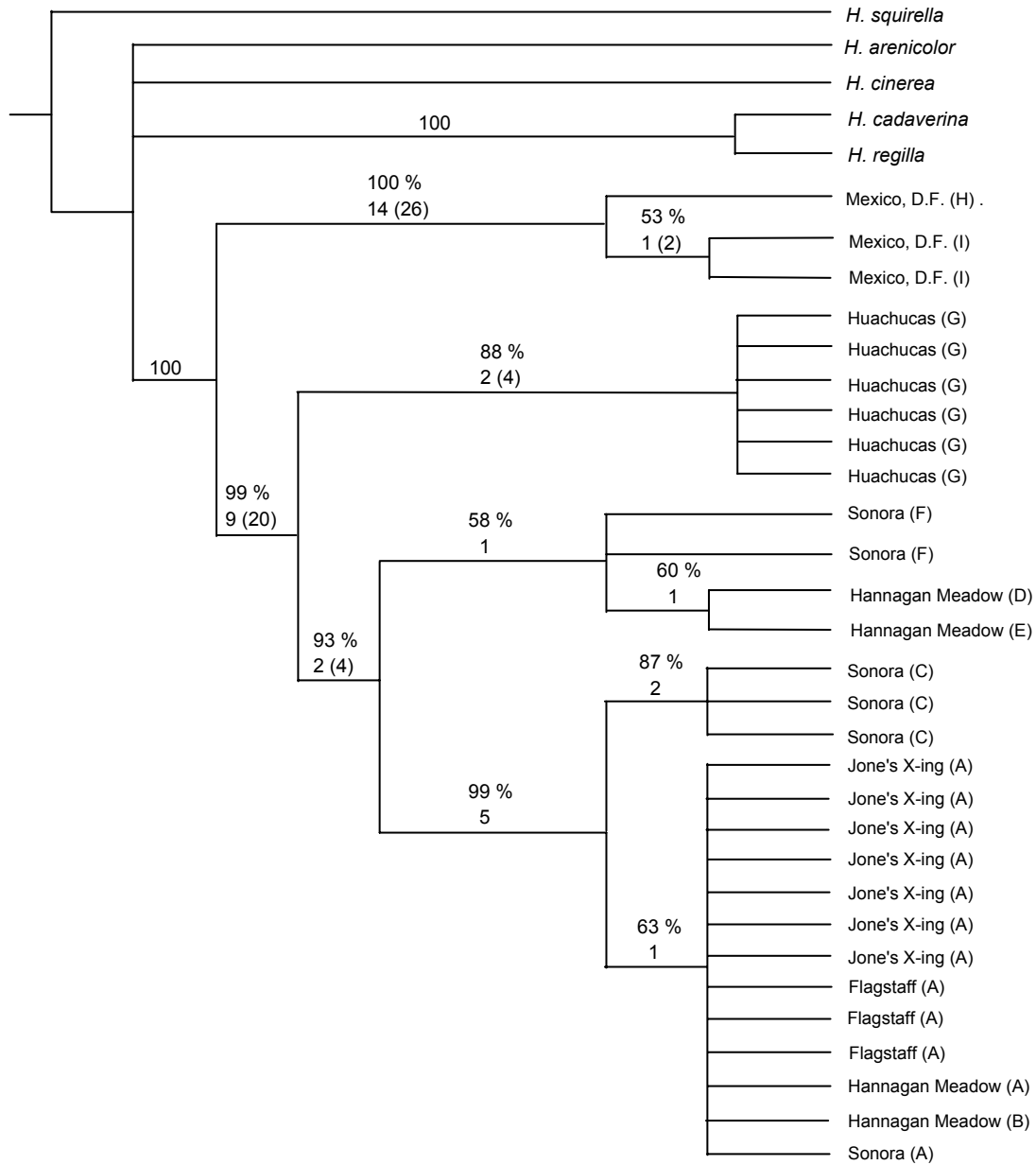


FIG. 5.--Single most parsimonious phylogeny of cytochrome *b* haplotypes (data analyzed with PAUP). Branch lengths at each node and bootstrap consensus value of branch are given at each node (for the *Hyla eximia* haplotype clade only). Identical haplotypes are given letter designations within parentheses. Samples of *Hyla arenicolor*, *H. cadaverina*, *H. cinerea*, *H. regilla*, and *H. squirella* were used for outgroup comparison.

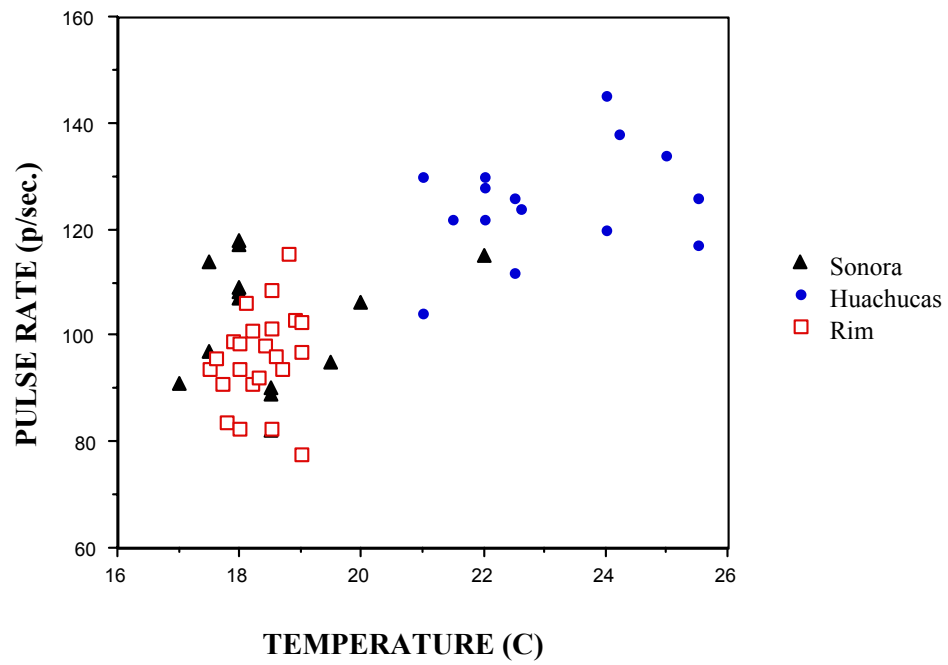


FIG. 6.--Advertisement call pulse rate plotted against cloacal temperature for Mogollon Rim, Huachucas, and Sonora populations.

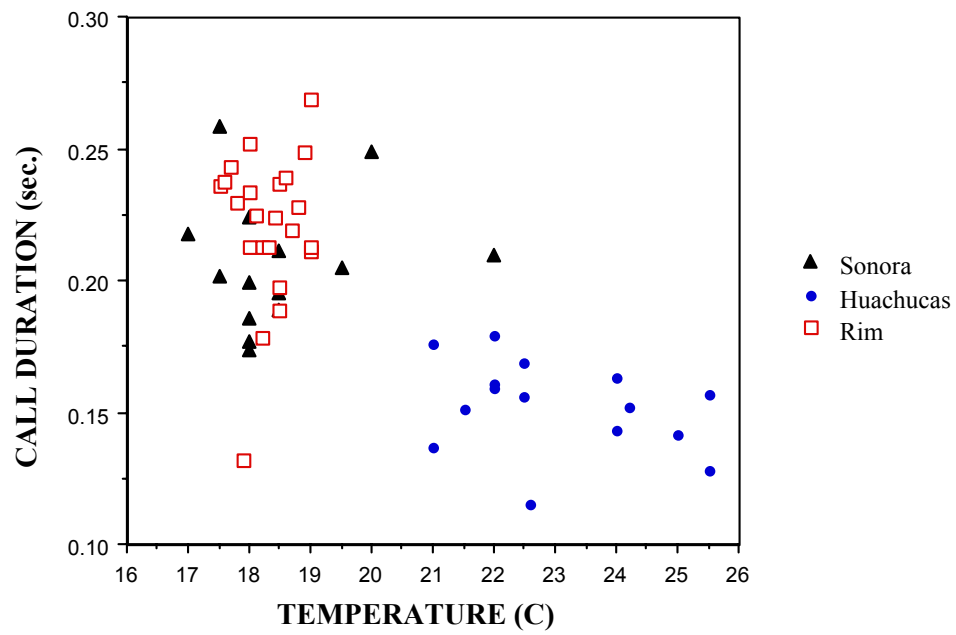


FIG. 7.--Call duration plotted against cloacal temperature for Mogollon Rim, Huachucas, and Sonora populations.

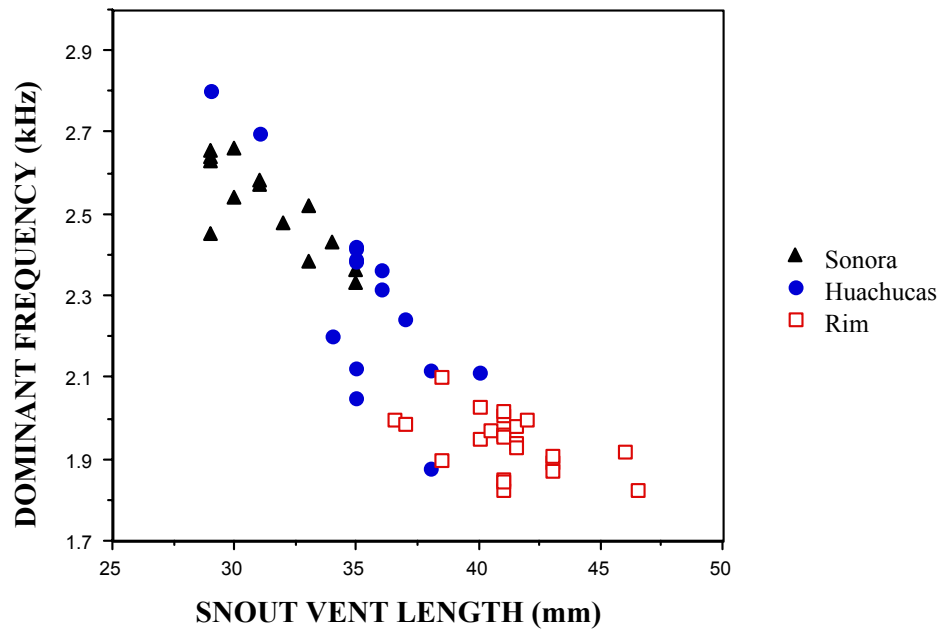


FIG. 8.--Advertisement call dominant frequency plotted against snout vent length for Mogollon Rim, Huachucas, and Sonora populations.